Structural Studies of Bacterial RNA Polymerase

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Abstract No. Dars9442
Beamline(s): **X25, X9B**

Introduction: Transcription is the major control point of gene expression and RNA polymerase (RNAP) is the central enzyme of transcription. Our long-term goal is to understand the mechanism of transcription and its regulation. Determining three-dimensional structure of RNAP and its complexes with DNA, RNA, and regulatory factors, is an essential step. This is best accomplished with highly characterized prokaryotic RNAPs, especially because of the high degree of conservation of RNAP structure and function from bacteria to man.

To this end, we recently determined the 3.3 Å-resolution crystal structure of a prokaryotic RNAP, the 380 kDa core RNAP from the thermophilic eubacteria *Thermus aquaticus* (Taq; subunit composition $\alpha_2\beta\beta'\omega$) and employed extensive crosslinking experiments to construct a model of the ternary elongation complex containing core RNAP, DNA template, and RNA transcript (1,2). Our current work is aimed towards adding to our understanding of the enzyme's function and its regulation

Results: We have two main lines of research, one on the RNAP itself (conducted mainly at X25), and on the promoter specificity σ factors (conducted mainly at X9B):

Structural studies of RNAP

1. We are investigating new crystallization conditions and mutant RNAPs to obtain crystals that diffract to higher resolution.

2. We are determining the 3.0 Å resolution crystal structure of Taq core RNAP complexed with nucleotide substrates.

3. We are determining the crystal structures of Taq core RNAP complexed with several antibiotic inhibitors.

4. We are determining the crystal structure of the 430 kDa Taq RNAP holoenzyme (core RNAP plus the 50 kDa Taq σ^A). So far we have promising crystals that diffract to 4.5 Å resolution.

5. We are determining the crystal structure of Taq RNAP holoenzyme complexed with a promoter DNA fragment. We have several promising crystal forms diffracting to near 4 Å resolution.

Structural studies of RNAP σ factors

1. In these studies, we are currently focusing on Taq σ^A. We have been unable to obtain crystals of any intact σ factor for many years, but we have obtained crystals of domains of Taq σ^A that span most of the important regions of the molecule. One crystal form contains a 9 kDa domain that comprises σ conserved region 4 (responsible for sequence-specific recognition of the conserved –35 promoter element and also a target for many transcription activators) that diffracts to 1.4 Å resolution. Another crystal form contains a fragment that spans σ conserved regions 1.2 to 3.1 and diffracts to 2.6 Å resolution.

2. We have promising crystals of a complex between an intact σ factor (the 29 kDa σ^F from *Bacillus stearothermophilus*) and its cognate regulatory molecule, the anti-σ factor SpoIIAB (a dimer of 16 kDa subunits). The crystals are very small (less than 50 μm in the longest dimension) but diffract to 3.5 Å resolution.

Conclusions: Our work in the past year has not resulted in any publications using data from X25 or X9B, but progress on these challenging projects has been good on many fronts.

Acknowledgments: Funding for these projects come from RO1 grants to S. Darst.

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